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(54) Method for preparing optically active 3-phenylglycidic acid esters.

(57) There is disclosed a method for preparing an optically active 3-phenylglycidic acid ester compound, which comprises permitting an enzyme having the ability of stereoselectively hydrolyzing an ester bond to act on a racemic 3-phenylglycidic acid ester which may be unsubstituted or substituted on the phenyl group, thereby stereoselectively hydrolyzing one of the optically active isomers and then separating and collecting the antipode from the reaction mixture.

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Method for preparing optically active 3-phenylglycidic acid esters

BACKGROUND OF THE INVENTION

This invention relates to a novel method for preparing optically active 3-phenylglycidic acid esters.

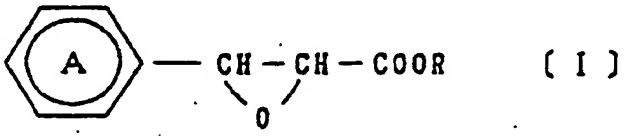
Optically active 3-phenylglycidic acid ester compounds are important ones as synthetic intermediates for dilthiazem hydrochloride useful as coronary vasodilator and other various pharmaceutical compounds, and in the prior art, as the method for preparing this compound, there has been known a method in which methyl trans-3-(4-methoxyphenyl)glycidate is hydrolyzed to a corresponding carboxylic acid, and the carboxylic acid is optically resolved with an optically active amine and then esterified (Japanese Unexamined Patent Publications Nos. 13775/1985 and 13776/1985).

However, the above method involves the drawback that many steps are included, and yet the desired optically active methyl trans-3-(4-methoxyphenyl)glycidate could be obtained only as an oil of low purity.

SUMMARY OF THE INVENTION

The present inventors have studied intensively in order to solve such drawback, and consequently found a method capable of obtaining the desired optically active 3-phenylglycidic acid ester compounds at once and as crystals from racemic 3-phenylglycidic acid ester compounds, to accomplish the present invention.

More specifically, according to the present invention, an optically active isomer of a 3-phenylglycidic acid ester compound of the formula:



[I]

wherein Ring A is a substituted or unsubstituted phenyl group, and R is an ester residue, which comprises

(a) permitting an enzyme having the ability of stereoselectively hydrolyzing an ester bond to act on a racemic 3-phenylglycidic acid ester compound (I), thereby stereoselectively hydrolyzing one of optically active isomers thereof, and then

(b) separating and collecting the antipode thereof from the reaction mixture.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is an IR absorption spectrum of methyl (2R, 3S)-3-(4-methoxyphenyl)glycidate; Fig. 2 a NMR spectrum of the same compound; and Fig. 3 a mass spectrum of the same compound.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Even when the 3-phenylglycidic acid ester compound represented by the formula [I] may have a substituent selected from a lower alkyl group, a lower alkoxy group and a halogen atom on the ring A, the method of the present invention can be practiced similarly to the case where no substituent exists on the ring A. Examples of such substituent may include methyl group, methoxy group or chloro atom at the 4-position. The ester residue R is ordinarily a lower alkyl group such as methyl, ethyl, isopropyl or t-butyl group.

In the present invention, as the racemic 3-phenylglycidic acid ester compound [I], not only one containing (2S, 3R) isomer and (2R, 3S) isomer in equal amounts, but any one which contains both of these optically active isomers can be used.

Examples of the enzyme to be used in the present invention include a group of hydrolases called lipase or esterase. These enzymes may be eithr those drived from microorganisms or those derived from animal cells, and further derived from vegetable cells. Also, they may be those extracted according to known methods from microorganism cells, animal cells or vegetable cells containing these hydrolases, or commercially available ones. Specific examples may include alkaline lipase (derived from *Achromobacter*, produced by Wako Junyaku), lipase (derived from *Chromobacterium* viscosum, produced by Toyo Jozo), lipase B (*Pseudomonas fragi* 22-39B, produced by Wako Junyaku), lipase M "Amano" 10 (derived from *Mucor javanicus*, produced by Amano Seiyaku), lipase type XI (derived from *Rhizopus arrhizus*, produced by Sigma, U.S.A.), talipase (derived from *Rhizopus delemar*, produced by Tanabe Seiyaku), lipase NK-116 (derived from *Rhizopus japonicus*, produced by Nagase Sangyo), lipase N (derived from *Rhizopus niveus*, produced by Amano Seiyaku), lipase type VII (derived from *Candida cylindracea*, produced by Sigma, U.S.A.), lipase (derived from porcine pancreas, produced by Wako Junyaku), esterase (porcine liver, produced by Sigma, U.S.A.), cholesterol esterase (derived from *Candida rugosa*, produced by Nagase Sangyo), etc.

In the present invention, a culture broth of a microorganism producing the above-mentioned enzyme, microbial cells collected from said culture broth and a processed product of said microbial cells can also be used as the enzyme source.

The microorganism which can be used in the present invention may be those having the ability of producing the hydrolase as mentioned above, and, for example, microorganisms such as molds, bacteria, yeasts and actinomycetes having such ability can be suitably used. Specifically, there may be included, as mold, the microorganisms belonging to the genus *Absidia*, the genus *Aspergillus*, the genus *Fusarium*, the genus *Gibberella*, the genus *Mucor*, the genus *Neurospora*, the genus *Trichoderma* or the genus *Rhizopus*; as bacterium the microorganisms belonging to the genus *Achromobacter*, the genus *Alcaligenes*, the genus *Bacillus*, the genus *Brevibacterium*, the genus *Corynebacterium*, the genus *Providencia*, the genus *Pseudomonas*, the genus *Serratia*; and as yeast the microorganisms belonging to the genus *Candida* or the genus *Saccharomycopsis*; and as actinomycete the microorganisms belonging to the genus *Nocardia*. Specific examples of such microorganisms may include, for example, *Absidia corymbifera* IFO 4009 (NRRL 2982), IFO 4010, *Aspergillus ochraceus* IFO 4346, *Aspergillus terreus* IFO 6123 (ATCC 10020), *Fusarium oxysporum* IFO 5942 and ATCC 659, *Fusarium solani* IFO 5232, *Gibberella fujikuroi* IFO 5268, *Mucor angulimacrosporus* IAM 6149, *Mucor circinelloides* IFO 6746 (ATCC 42258), *Mucor flavus* IAM 6143, *Mucor fragilis* IFO 6449, *Mucor genevensis* IAM 6091, *Mucor globosus* IFO 6745, *Mucor hiemalis* OUT 1045 and OUT 1047, *Mucor janssenii* OUT 1050 and IFO 5398, *Mucor javanicus* IFO 4569, IFO 4570, IFO 4572 and IFO 4382, *Mucor lamprosporus* IFO 6337, *Mucor petrinsularis* IFO 6751, *Mucor plumbeus* IAM 6117, *Mucor praini* IAM 6120, *Mucor pusillus* IAM 6122, *Mucor racemosus* IFO 4581, *Mucor ramannianus* IAM 6128, Mucor recurvus IAM 6129, *Mucor silvaticus* IFO 6753, *Mucor spinescens* IAM 6071, *Mucor subtilissimus* IFO 6338, *Neurospora crassa* IFO 6068 (ATCC 10336), *Rhizopus arrhizus* IFO 5780 (ATCC 11145), *Rhizopus delemar* ATCC 34612, *Rizopus japonicus* IFO 4758, *Trichoderma viride* OUT 4208 and IFO 4847, *Achromobacter cycloclastes* IAM 1013, *Alcaligenes faecalis* OUT 8030 (ACC 106), *Bacillus sphaericus* IFO 3525 (ATCC 10208), *Bacillus subtilis* OUT 8104 and OUT 8106, *Brevibacterium ketoglutamicum* ATCC 15588, *Corynebacterium alkanolyticum* ATCC 21511, *Corynebacterium hydrocarboclastum* ATCC 15592, *Corynebacterium primorioxydans* ATCC 31015, *Providencia alcalifaciens* JCM 1673 (ATCC 9886), *Pseudomonas mutabilis* ATCC 31014, *Pseudomonas putida* ATCC 17426, ATCC 17453 and ATCC 33015, *Serratia liquefaciens* ATCC 27592, *Serratia marcescens* ATCC 13880, ATCC 14764, ATCC 19180, ATCC 21074, ATCC 27117 and ATCC 21212, *Candida parapsilosis* IFO 0585, *Saccharomycopsis lipolytica* IFO 0717, IFO 0746 (NRRL Y-1095), IFO 1195, IFO 1209 (NRRL Y-1094) and IFO 1548, *Nicardia asteroides* IFO 3384 (ATCC 330), IFO 3424 and IFO 3423, *Nocardia gardneri* ATCC 9604, etc. These may be either wild species or mutant strains, and further may be those derived from these microorganisms according to the bioengineering methods such as gene recombination, cell fusion, etc.

Also, the culture broth and microbial cells of the above microorganism can be obtained by cultivating said microorganism in a medium conventionally used in this field of the art, for example, a medium containing carbon sources, nitrogen sources and inorganic salts conventionally used, at room temperature or under heating (preferably about 20 to 40 °C), and under aerobic conditions at pH of about 5 to 8 and, if necessary, separating and collecting the cells from the culture broth in conventional manner.

During cultivation, it is also possible to enhance the enzyme activity by adding the racemic 3-phenylglycidic acid ester compound (I) into the medium in an amount of about 0.001% or more, particularly about 0.1 to 1%.

As the processed product of such microbial cells, there may be included lyophilized cells, acetone

dried cells, self-digested product of cells, cell extract, cell ground product, sonication treated product of cells of the above microorganism. Further, the microbial cells or processed product of cells of the present invention can be immobilized according to the known method such as the polyacrylamide method, the sulfur containing polysaccharide gel method (carrageenan gel method), the alginic acid gel method, the agar gel method, etc. before use.

The stereoselective hydrolysis reaction according to the present invention can be practiced by contacting the enzyme with a racemic 3-phenylglycidic acid ester compound [I] in an appropriate solvent.

The substrate concentration may be preferably 0.05 to 20%, above all 0.5 to 5%, and the reaction will suitably proceed at room temperature or under heating, preferably at 10 to 50 °C, particularly preferably 25 to 40 °C. During the reaction, it is preferable to adjust the pH of the reaction mixture to pH 5 to 10, paticularly 6 to 9. In this case, since most of the substrates are difficultly soluble in water, it is preferable to carry out the reaction in a two-phase solvent system of water or an aqueous solvent and an organic solvent. Examples of such organic solvent may include carbon tetrachloride, chloroform, dichloromethane, trichloroethylene, chlorobenzene, benzene, toluene, xylene, t-butyl methyl ether, diisopropyl ether, diethyl ether, methyl isobutyl ketone, methyl ethyl ketone, ethyl acetate, butyl acetate, n-propyl alcohol, isopropyl alcohol, n-butyl alcohol, t-butyl alcohol, etc., particularly preferably ethyl acetate, carbon tetrachloride and toluene. When the culture broth or the microbial cells are used as the enzyme source, it is preferred to carry out the above reaction in the presence of a surfactant in order to shorten the reaction time or increase yield of the optically active 3-phenylglycidic acid ester compound [I]. As such surfactant, cetylpyridinium bromide, cetyltrimethyl-ammonium bromide, polyethylene glycol, polyoxyethylene octylphenyl ether, etc. can be used, and its amount added may be preferably about 0.0001 to 0.1% based on the reaction mixture.

Isolation of the optically active 3-phenylglycidic acid ester compound [I] from the reaction mixture thus obtained can be easily practiced according to the conventional method. For example, when hydrolysis reaction is carried out in a water-organic solvent two-phase system, one optically active isomer of the 3-phenylglycidic acid ester compound [I] is hydrolyzed to be migrated into the aqueous layer, while the other optically active isomer not subjected to the reaction remains in the organic solvent, and therefore the optically active 3-phenylglycidic acid ester compound can be collected as crystals by separating the organic solvent layer and subjecting it to concentration under reduced pressure.

According to the present invention, the optically active 3-phenylglycidic acid ester compound [I] can be obtained in short steps and yet as crystals of high purity, and therefore the method can be a commercially advantageous preparation method.

Example 1

Fifty (50) ml of a medium (pH 7.0) containing 0.5% glucose, 1% peptone, 1% meat extract, 1.25% yeast extract and 0.5% sodium chloride were placed in a 500 ml volume shaking flask and sterilized at 120 °C for 10 minutes. Into the medium, one platinum loop of *Serratia marcescens* ATCC 27117 was inoculated, and cultivation was performed under shaking at 30 °C for 20 hours. The microbial cells collected by centrifugation from 5.4 liters of the above culture were suspended in physiological saline and further the microbial cells were collected by centrifuga-tion. The microbial cells were suspended in 1.8 liters of 10 mM phosphate buffer (pH 7.5) containing 0.01% cetyltrimethylammonium bromide, and added into 1.8 liters of carbon tetrachloride containing 7.2 g of racemic methyl 3-(4-methoxyphenyl)glycidate. Then, the stereoselective hydrolysis reaction was carried out at 30 °C for 3 days, whereby methyl (2S, 3R)-3-(4-methoxyphenyl)-glycidate was completely hydrolyzed. The carbon tetra-choride layer was separated, then concentrated under reduced pressure to obtain 3.0 g of methyl (2R, 3S)-3-(4-methoxyphenyl)glycidate as crude crystals. After 10 ml of isopropyl alcohol was added to 3.0 g of the crude crystals, the mixture was dissolved by heating at 80 °C under stirring for 20 minutes. After gradually cooled from 80 °C to 20 °C over 3 hours, the mixture was ice-cooled for one hour and the precipitated crystals were filtered to obtain 2.9 g of crystals of methyl (2R, 3S)-3-(4-methoxyphenyl)glycidate.

M.P.: 87 - 88 °C

$[\alpha]_D^{20}$: -207.08° (C = 1, methanol)

Purity: 100%

Elemental : Calcd.	C: 63.45,	H: 5.81,	O: 30.74
analysis Found	C: 63.44,	H: 5.80,	O: 30.76

IR spectrum: Fig. 1
NMR spectrum: Fig. 2
Mass spectrum: Fig. 3

Example 2

Into the medium shown in Example 1 was inoculated each of the bacteria shown in Table 1, and cultured at 30 °C for 20 hours. The microbial cells collected from 45 ml of the above medium by centrifugation were suspended in physiological saline and then the cells were further collected by centrifugation. Said cells were suspended in 15 ml of 10 mM phosphate buffer (pH 7.5) containing 0.01% cetyltrimethylammonium bromide, and added into 15 ml of carbon tetrachloride containing 60 mg of racemic methyl 3-(4-methoxyphenyl)glycidate to carry out stereoselective hydrolysis at 30 °C for 3 days. After the reaction, the carbon tetrachloride layer was separated to obtain a reaction mixture containing methyl (2R, 3S)-3-(4-methoxyphenyl)glycidate. The content of the (2R, 3S) isomer in the reaction mixture was as shown in Table 1, and substantially no (2S, 3R) isomer which is its antipode was detected in the reaction mixture.

Quantitation of the above optical isomer was conducted by high performance liquid chromatography by use of a chiral cell OJΦ 4.6 x 250 mm manufactured by Dicel Kagaku Kogyo K.K. (hereinafter the same).

Table 1

Microorganism used	(2R, 3S) isomer content (mg/ml)
Achromobacter cycloclastes IAM 1013	0.8
Alcaligenes faecalis OUT 8030	0.7
Bacillus sphaericus IFO 3525	0.7
Bacillus subtilis OUT 8104	0.8
Brevibacterium ketoglutamicum ATCC 15588	0.7
Corynebacterium alkanolyticum ATCC 21511	1.8
Corynebacterium primorioxidans ATCC 31015	1.8
Providencia alcalifaciens JCM 1673	1.8
Pseudomonas mutabilis ATCC 31014	1.8
Pseudomonas putida ATCC 17453	1.8
Serratia liquefaciens ATCC 27592	1.6
Serratia marcescens ATCC 27117	1.8

Example 3

Fifty (50) ml of a medium (pH 6.2) containing 1% glucose, 0.5% peptone, 0.3% yeat extract, 0.3% malt extract were placed in a 500 ml volume shaking flask, and sterilized at 120 °C for 10 minutes. Into this medium was inoculated one platinum loop of mold or yeast shown in Table 2, and mold was cultured at 27 °C for 68 hours under shaking, and yeast at 27 °C for 20 hours at under shaking. Into 45 ml of the above culture broth was added 15 ml of carbon tetrachloride containing 60 mg of racemic methyl 3-(4-methoxyphenyl)glycidate, and further cetyltrimethylammonium bromide was added in such an amount as to give a concentration of 0.001% in the reaction mixture, to carry out the stereoselective hydrolysis reaction at 30 °C for 3 days. After the reaction, the carbon tetrachloride layer was separated to obtain a reaction mixture containing methyl (2R,3S)-3-(4-methoxyphenyl)glycidate. The content of the (2R, 3S) isomer in the reaction mixture was as shown in Table 2, and substantially no (2S, 3R) isomer which is its antipode was detected in the reaction mixture.

Table 2

Microorganism used	(2R, 3S) isomer content (mg/ml)
Absidia corymbifera IFO 4010	0.8
Asperigillus ochraeus IFO 4346	0.8
Asperigillus terreus IFO 6123	1.3
Fusarium oxysporum IFO 5942	0.8
Fusarium solani IFO 5232	0.7
Gibberella fujikuroi IFO 5268	0.8
Mucor globosus, IFO 6745	1.8
Mucor javanicus IFO 4572	1.8
Mucor lamprosporus IFO 6337	1.8
Mucor silvaticus IFO 6753	1.8
Neurospora crassa IFO 6068	0.7
Rhizopus arrhizus IFO 5780	1.8
Rhizopus japonicus IFO 4758	1.8
Trichoderma viride OUT 4208	0.7
Candida parapsilosis IFO 0585	0.8
Saccharomycopsis lipolytica IFO 0717	1.8

Example 4

Fifty (50) ml of a medium (pH 7.3) containing 0.4% glucose, 0.4% yeast extract and 1.0% malt extract were placed in a 500 ml volume shaking flask, and sterilized at 120 °C for 10 minutes. Into this medium, one platinum loop of the actinomycetes shown in Table 3 was inoculated, and cultivation was conducted under shaking at 27 °C for 68 hours. Into 45 ml of the above culture broth was added 15 ml of carbon tetrachloride containing 60 mg of racemic methyl 3-(4-methoxyphenyl)glycidate, and further cetyltrimethylammonium bromide was added in such an amount as to give a concentration of 0.001% in the reaction mixture, to carry out the stereoselective hydrolysis reaction at 30 °C for 3 days. After the reaction, the carbon tetrachloride layer was separated to obtain a reaction mixture containing methyl (2R, 3S)-3-(4-methoxyphenyl)glycidate. The (2R, 3S)-isomer in the reaction mixture is as shown in Table 3, and substantially no (2S, 3R)-isomer which is its antipode was detected in the reaction mixture.

Table 3

Microorganism used	(2R, 3S) isomer content (mg/ml)
Nocardia asteroides IFO 3384	1.3
Nocardia gardneri ATCC 9604	0.8

Example 5

After each lipase or esterase enzyme shown in Table 4 was suspended in 1 ml of 10 mM phosphate buffer adjusted to the optimum pH for each enzyme, 1 ml of carbon tetrachloride containing 4 mg of racemic methyl 3-(4-methoxyphenyl)glycidate was added, and the stereoselective hydrolysis reaction was

carried out at 30 °C for 2 days. After the reaction, carbon tetrachloride layer was separated to obtain a reaction mixture containing methyl (2R, 3S)-3-(4-methoxyphenyl)glycidate. The content of the (2R, 3S) isomer in the reaction mixture was as shown in Table 4, and substantially no (2S, 3R)-isomer which is its antipode was detected in the reaction mixture.

Table 4

Enzyme used (100 units)		(2R, 3S)-isomer content (mg/ml)
Alkaline lipase	(derived from *Achromobacter*, manufactured by Wako Junyaku)	0.7
Lipase	(derived from *Chromobacterium viscosum*, manufactured by Toyo Jozo)	0.7
Lipase B	(derived from *Pseudomonas flagi* 22-39B, manufactured by Wako Junyaku)	0.7
Lipase M "Amano" 10	(derived from *Mucor javanicus*, manufactured by Amano Seiyaku)	1.6
Lipase type XI	(derived from *Rhizopus arrhizus*, manufactured by Sigma, U.S.A)	1.8
Talipase	(derived from *Rhizopus delemar*, manufactured by Tanabe Seiyaku)	1.8
Lipase NK-116	(derived from *Rhizopus japonicus*, manufactured by Nagase Sangyo)	1.8
Lipase	(derived from *Rhizopus nibius*, manufactured by Nagase Sangyo)	1.8
Lipase type VII	(derived from *Candida cylindracia*, manufactured by Sigma)	1.6
Lipase	(derived from porcine pancreas, manufactured by Wako Junyaku)	0.7

Table 4 (cont'd)

Enzyme used (100 units)		(2R, 3S)-isomer content (mg/ml)
Esterase	(derived from porcine liver, manufactured by Sigma, U.S.A)	0.7
Cholesterol esterase	(derived from *Candida rugosa*, manufactured by Nagase Sangyo)	1.5

Example 6

After each lipase enzyme (10 mg) shown in Table 5 was suspended in 1 ml of 100 mM phosphate buffer adjusted to the optimum pH for each enzyme, 1 ml of carbon tetrachloride containing 4 mg of racemic methyl 3-(4-methoxyphenyl)glycidate was added, to carry out the stereoselective hydrolysis reaction at 30 °C for one day. After the reaction, carbon tetrachloride layer was separated to obtain a reaction mixture containing methyl (2R, 3S)-3-(4-methoxyphenyl)glycidate. The content of the (2R, 3S) isomer in the reaction mixture was as shown in Table 5, and substantially no (2S, 3R)isomer which is its antipode was detected in the reaction mixture.

Table 5

Enzyme used (10 mg/ml)	(2R, 3S) isomer content (mg/ml)
Palatase A (derived from *Aspergillus niger*, manufactured by Novo)	1.2
Lipase AY (derived from *Candida cylindracea*, manufactured by Amano Seiyaku)	1.3
Lipase OF-360 (derived from *Candida cylindracea*, manufactured by Meito Sangyo)	1.8
Lipase CE (derived from *Humicola lannginosa*, manufactured by Amano Seiyaku)	1.2
Palatase M (derived from *Mucor miehei*, manufactured by Novo)	1.7
Lipase P (derived from *Pseudomonas fluorescens*, manufactured by Amano Seiyaku)	1.6
Lipase CES (derived from *Pseudomonas sp*, manufactured by Amano Seiyaku)	1.7
Lipase YS (derived from *Pseudomonas sp*, manufactured by Amano Seiyaku)	1.3
Lipase (derived from *Rhizopus chinensis*, manufactured by Yukijirushi)	1.2
Lipase(Saiken)100 (derived from *Rhizopus japonicus* manufactured by Nagase Sangyo)	1.8
Lipase P (derived from *Rhizopus javanicus*, manufactured by Amano Seiyaku)	1.2
Lipase B (derived from *Pseudomonas frag*:22-39B manufactured by Wako Junyaku)	1.2
Lipase LP (derived from *Chromobacterium viscosum*, manufactured by Toyo Jozo)	0.7
Lipase M (derived from *Mucor javanicus*, manufactured by Amano Seiyaku)	1.7
Talipase (derived from *Rhizopus delemar*, manufactured by Tanabe Seiyaku)	1.6
Lipase N (derived from *Rhizopus niveus*, manufactured by Amano Seiyaku)	1.6

Example 7

Into 500 ml of 0.5 M phosphate buffer (pH 7.5) containing 5g/L lipase OF-360 (drived from Cardida

cylindracea, manufactured by Meito Sangyo) were added 500 ml of toluene containing 100 g of racemic methyl 3-(4-methoxyphenyl)glycidate, and then the aqueous phase and the toluene phase were mixed at a stirring rate of 600 rpm to carry out the stereoselective hydrolysis reaction for 6 hours. After the reaction, the toluene layer was separated then concentrated under reduced pressure to obtain 42.5 g of methyl (2R, 3S)-3-(4-methoxyphenyl)-glycidate as crude crystals. After adding 140 ml of isopropyl alcohol to 42.5 g of these crude crystals, the mixture was dissolved by heating at 80 °C under stirring for 20 minutes. After gradually cooled from 80 °C to 20 °C over 3 hours, the mixture was ice-cooled for one hour and the precipitated crystals were filtered to obtain 40.2 g of crystals of methyl (2R, 3S)-3-(4-methoxyphenyl)-glycidate.

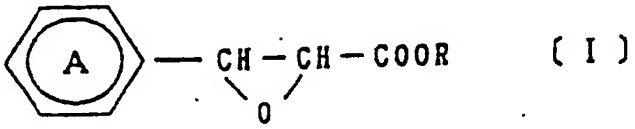
M.P.: 87 - 88 °C

$[\alpha]_D^{20}$: -206.4° (C = 1, methanol)

Purity: >99%

Claims

1. A method for preparing an optically active isomer of a 3-phenylglycidic acid ester compound of the formula:



(I)

wherein Ring A is a substituted or unsubstituted phenyl group, and R is an ester residue, which comprises

(a) permitting an enzyme having the ability of stereoselectively hydrolyzing an ester bond to act on a racemic 3-phenylglycidic acid ester compound (I), thereby stereoselectively hydrolyzing one of optically active isomers thereof, and

(b) separating and collecting the antipode thereof from the reaction mixture.

2. The method according to Claim 1, wherein the enzyme is esterase or lipase.

3. The method according to claim 1, wherein the configuration of the antipode to be separated and collected is (2R, 3S).

4. The method according to claim 1, wherein the antipode to be separated and collected is a lower alkyl (2R, 3S)-3-(4-methoxyphenyl)glycidate.

5. The method according to claim 1, wherein the antipode to be separated and collected is methyl (2R, 3S)-3-(4-methoxyphenyl)glycidate.

6. The method according to Claim 1, wherein the stereoselective hydrolysis reaction is practiced by contacting the enzyme with the racemic 3-phenylglycidic acid ester compound in a solvent.

7. The method according to Claim 2, wherin a culture broth of a microorganism producing said enzyme, microbial cells collected from said culture broth or a processed product of said microbial cells is used as the enzyme source.

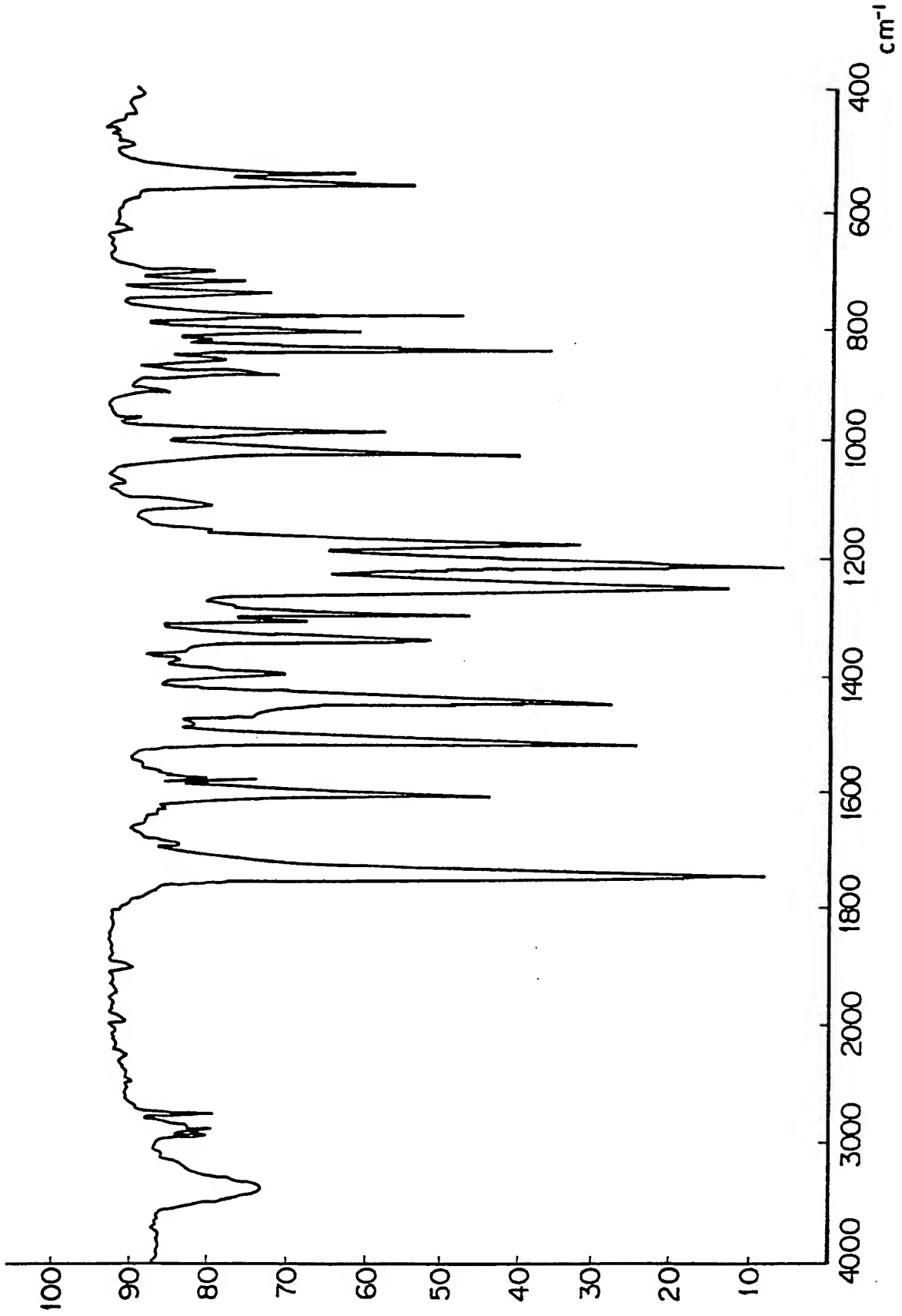
8. The method according to claim 7, wherein the microorganism is a micororganism belonging to the genus *Absidia*, the genus *Asperigillus*, the genus Fusarium, the genus *Gibberella*, the genus *Mucor*, the genus *Neurospora*, the genus *Rhizopus*, the genus *Trichoderma*, the genus *Achromobacter*, the genus *Alcaligenes*, the genus *Bacillus*, the genus *Brevibacterium*, the genus *Corynebacterium*, the genus *Providencia*, the genus *Pseudomonas*, the genus *Serratia*, the genus *Candida*, the genus *Saccharomycopsis* or the genus *Nocardida*.

9. The method according to claim 6, wherein substrate concentration is 0.05 to 20%.

10. The method according to claim 6, wherein the reaction is practised in a two-phase solvent system of water or an aqueous solvent and an organic solvent.

11. The method according to claim 10, wherein the organic solvent is selected from the group consisting of carbon tetrachloride, chloroform, dichloromethane, trichloroethylene, chlorobenzene, benzene, toluene, xylene, t-butyl methyl ether, diisopropyl ether, diethyl ether, methyl isobutyl ketone, methyl ethyl ketone, ethyl acetate, butyl acetate, n-propyl alcohol, isopropyl alcohol, n-butyl alcohol and t-butyl alcohol.

FIG. 1



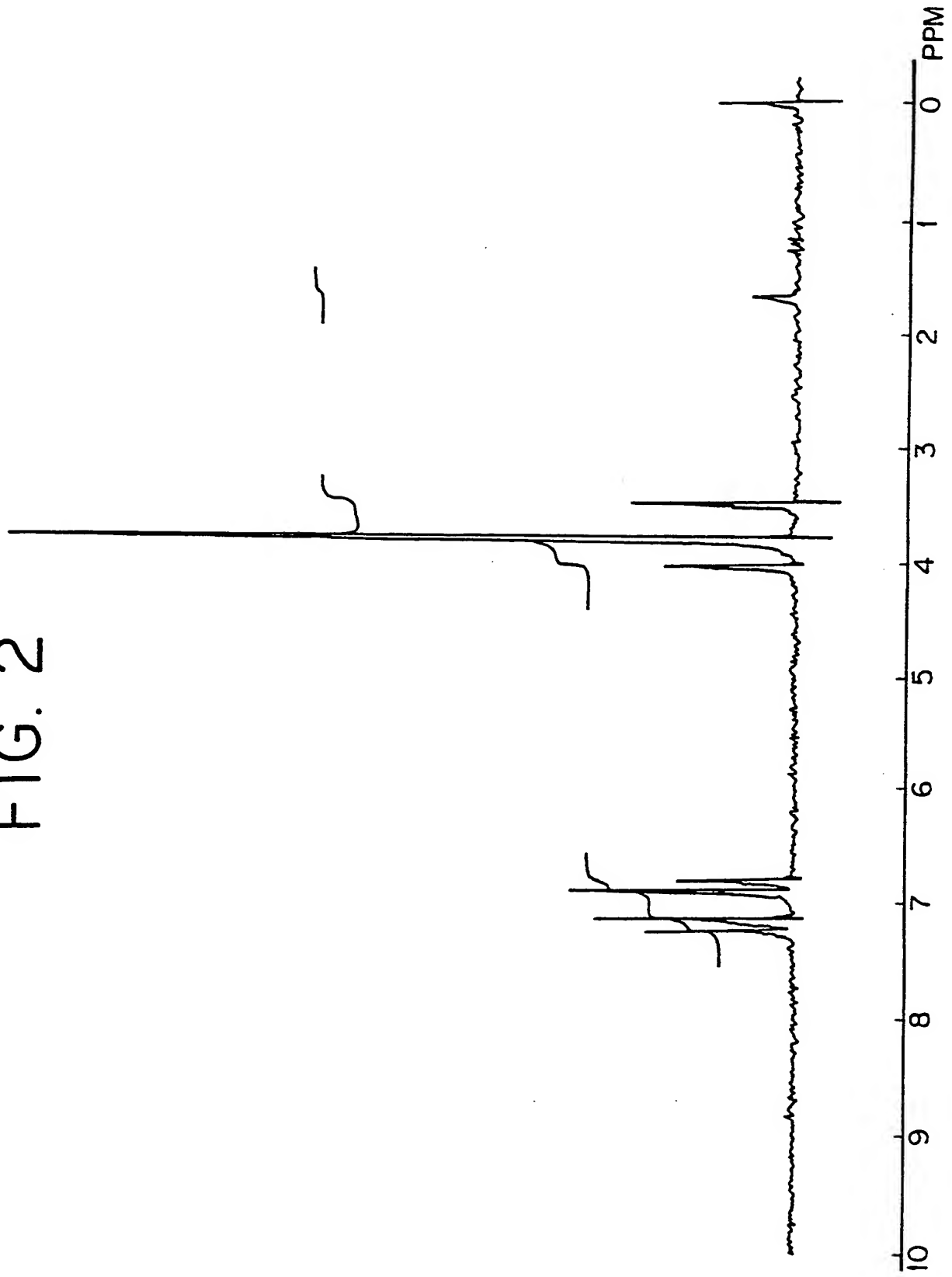
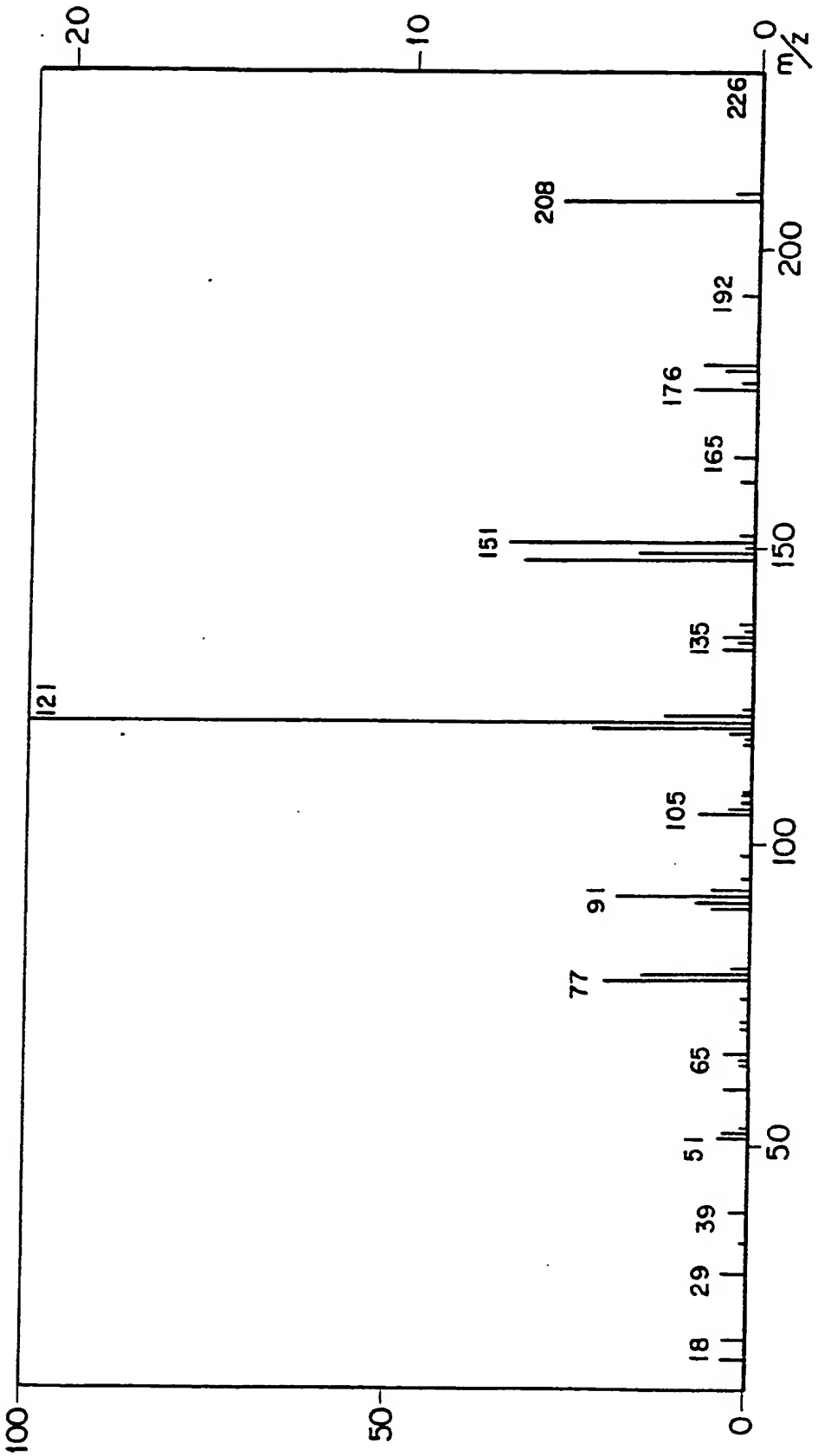


FIG. 2

FIG. 3



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EUROPEAN SEARCH REPORT

Application Number

EP 89 11 6104

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	EP-A-0 158 339 (TANABE SEIYAKU) * Claims; pages 13,14 * ---	1	C 12 P 41/00 C 12 P 17/02 C 07 D 303/48
A	EP-A-0 237 983 (INSTITUTO GUIDO DONEGANI) * Claims; pages 3,4 * ---	1	
A	EP-A-0 264 457 (SUMITOMO) * Claims * ---	1	
A	TETRAHEDRON, vol. 41, no. 7, 1985, pages 1393-1399, Pergamon Press Ltd , GB; P. MELLONI et al.: "Configurational studies on 2-[alpha-(2-ethoxyphenoxy)benzyl]morpholine FCE 20124" * Pages 1394,1395 * -----	1	

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

C 12 P
C 07 D

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	06-12-1989	DELANGHE L.L.M.

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